

# Thiol Esters of 2-Mercaptoethanol and 3-Mercapto-1,2-Propanediol

2-Mercaptoethanol and 3-mercapto-1,2-propanediol are selectively S-acylated by fatty acids with dicyclohexylcarbodiimide (DCC) catalyzed by 4-dimethylaminopyridine. Since reaction of such thiols with acid halides produces mixtures of S- and O-acylated materials, the procedure using DCC is more efficient. Such thiol esters are valuable as substrates in the study of lipase activity, using Ellman's Reagent in a continuous spectrophotometric assay. This selective synthesis will facilitate the study of fatty acid residues that are less available. Rearrangement of S- to O-acylated compounds occurs only slowly under the conditions of the lipase assay and should not significantly affect activity measurements. Using DCC, it is possible to sequentially esterify 2-mercaptoethanol to form unsymmetrical diesters.

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There is continuing interest in the chemistry and characterization of lipases. Jensen recently critically reviewed the methods for detecting and determining the triacylglycerol hydrolases (EC 3.1.1.3) (1). In 1976, Aarsman *et al.* reported the use of thiol esters as substrates for spectrophotometric assays of thiolate ion (2). This technique relies upon the nucleophilicity of thiol released, for example, by hydrolysis toward Ellman's Reagent (5,5'-dithio-bis[2-nitrobenzoic] acid) to produce a chromophore with  $\lambda_{\max} = 410$ . By employing solutions of hexamethylphosphoric triamide (HMPT), the relatively insoluble thiol esters of long chain fatty acids, such as 2-mercaptoethanol palmitate, can be dissolved, thereby extending the use of Ellman's Reagent to evaluate lipase activity in a homogeneous medium (3). The utility of this assay method would be extended greatly if the preparation of the thiol esters—that also contain one or more hydroxyl groups—could be improved. The published procedure involves treating ethereal solutions of the mercaptoalcohols with the required acid halide and pyridine. The hydroxyl and thiol groups are competitively acylated, and the resulting mixture is separated into the component monoacylated adducts and the diester by column chromatography. In order to facilitate the study of candidate substrates, the acid residues, which are themselves the products of synthesis, a more efficient procedure seemed desirable. Recently, glycidol esters have been converted to 1-S analogs of 1,3-diacylglycerols using thiolacids (4); the chemistry reported here offers a complementary approach with broader applicability.

In addition, although the ease of transacylation in the case of vic-diols is well understood (5), and studies of the related N-O rearrangements also have been discussed recently (6), only limited information about analogous S-O migrations have been reported, at least in simple aliphatic systems (4). Such transacylations in the case of  $\beta$ -hydroxythiol esters during lipase assays could cause erroneously elevated levels of lipase activity. In any case, it seemed useful to establish the stability of such thiol esters toward the usual conditions for preparing and purifying organic compounds.

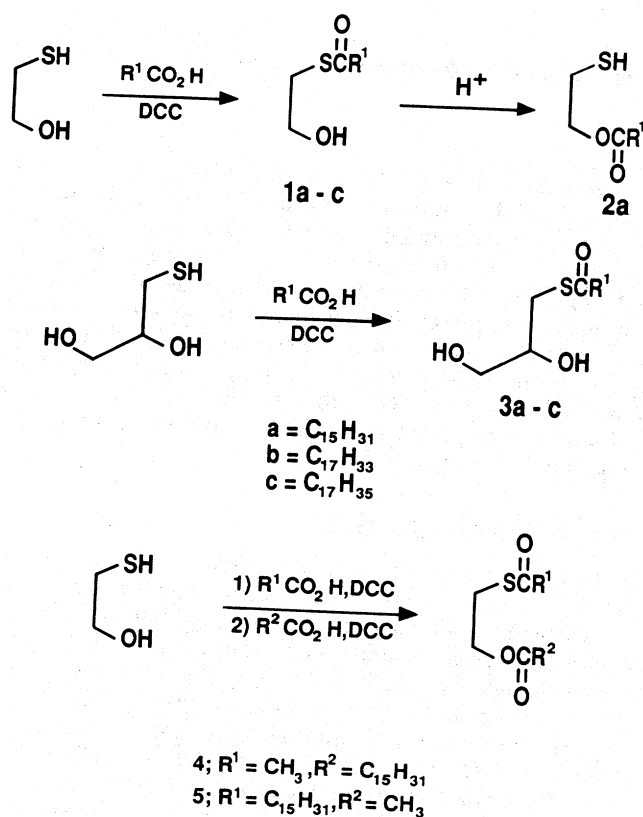
## MATERIALS AND METHODS

Nuclear magnetic resonance spectra were obtained ( $^{13}\text{C}$  NMR) with a Bruker 300 MHz spectrometer; only diagnostically useful signals are recorded below. Infrared spectra (IR) were obtained on a Perkin-Elmer 1310 Spectrophotometer (Norwalk, CT) using 3% solutions in carbon tetrachloride. Gas liquid chromatography (GLC) was accomplished using a Shimadzu GC-Mini 2 instrument (Columbia, MD) using either an SP-2340 or SPB-1, column (0.25 mm i.d.  $\times$  30 m) with flame ion detection, and He as carrier gas with a 50:1 split ratio. Mass spectra (GC-MS) were obtained with a Hewlett-Packard 5995 GC-MS system (Avondale, PA) that was interfaced with an OV-1 column (0.25 mm  $\times$  12 m). Chemical analyses were accomplished by Microanalysis Inc. (Wilmington, DE). Thin layer chromatography (TLC) was done using silica gel G (0.25 mm) from Analabs Inc. (Radnor, PA).

All organic solvents were reagent grade or better. The fatty acids employed were obtained from Aldrich Chem. Co. (Milwaukee, WI) and were >96% pure; the oleic acid was a gift of Nippon Oil Co. (Nagoya, Japan) and was 99% pure. The mercaptoalcohols, dicyclohexylcarbodiimide (DCC), and 4-dimethylaminopyridine (DMAP) were also purchased from Aldrich Chem. Co., and were employed directly.

**2-Mercaptoethanol S-alkanoates (1).** The alkanolic acid (10 mmol) was added to a solution of DCC (2.18 g, 10.5 mmol), 2-mercaptoethanol (0.75 ml, 10.7 mmol), and DMAP (0.12 g, 1.0 mmol) in 100 ml of methylene chloride that was stirred in an ice bath. The mixture was stirred for 2 hr without the cooling bath. The reaction mixture was suction filtered, and the filtrate was shaken with 100 ml of 2N HCl and separated. The organic phase was thoroughly washed with water, dried ( $\text{MgSO}_4$ ), and then stripped of solvent. The crude product was taken up in hot hexane (about 50 ml per 2 g of crude product) and filtered to remove residual urea. The solution was cooled ( $-20^\circ\text{C}$ ) and suction filtered to obtain the crystallized thioalcohol. In this manner, the following was obtained:

S-Palmitate (1a; 72.5%): mp  $57-59^\circ\text{C}$ ; IR 3460, 3400 (br), 1690, 1050  $\text{cm}^{-1}$ ;  $^{13}\text{C}$  NMR 200.1 (C=O), 61.94 ( $\text{CH}_2\text{H}$ ), and 44.15 ( $\text{CH}_2\text{SC=O}$ ) ppm GLC (SPB-1,  $260^\circ\text{C}$ )  $k' = 2.28$  (O-ester  $k' = 0.84$ ); TLC (90:10  $\text{CHCl}_3$ -



acetone)  $R_f = 0.36$  (O-ester  $R_f = 0.78$ ). Anal. Calcd. for  $C_{18}H_{36}O_2S$ : C, 68.30; H, 11.46; S, 10.13. Found: C, 68.57; H, 11.80; S, 10.32.

S-Oleate (**1b**; 90%): Liquid at 25°C; spectral and TLC data as for the palmitate; and additional signals in the  $^{13}C$  NMR for the alkene carbons at 129.6 and 129.9 ppm; GLC (SPB-1, 260°C)  $k' = 3.7$  (O-ester  $k' = 1.4$ ).

S-Stearate (**1c**; 67%): mp 63.5–64.5°C; spectral and TLC data as for the palmitate; GLC (SPB-1, 260°C)  $k' = 4.0$  (O-ester  $k' = 1.5$ ).

3-Mercapto-1,2-propanediol S-alkanoates (**3**). These S-esters were prepared in the same manner as the S-esters of 2-mercaptoethanol.

S-Palmitate (**3a**; 52%): mp 76–77°C (methanol); IR 3640, 3400 (br), 1690, 1050  $cm^{-1}$ ;  $^{13}C$  NMR 201.04 (C=O), 71.28 and 64.63 (C-O), and 44.11 (C-S) ppm; TLC (90:10  $CHCl_3$ -acetone)  $R_f = 0.2$  (the O-ester isomers are 0.58 for secondary alcohol ester and 0.83 for the primary ester).

S-Oleate (**3b**; 60%): mp 48–50°C; spectral and chromatographic data as for the palmitate and additional signals in the  $^{13}C$  NMR for the alkene carbons at 129.6 and 129.9 ppm.

S-Stearate (**3c**; 63%): mp 78.5–81°C; spectral and chromatographic data as for the palmitate.

2-Mercaptoethanol *O*-palmitate (**2a**). The S-palmitate ester (0.24 g, 0.76 mmol) was warmed under gentle reflux in benzene (20 ml) containing 30 mg of *p*-toluene-sulfonic

acid (anhydrous) for 16 hr. The solvent was removed, and the crude product was recrystallized from hexane with filtration of the hot solution to give the O-ester: 0.16 g, 67%, mp 38–39°C; IR 1740, 1170  $cm^{-1}$ ;  $^{13}C$  NMR 173.54 (C=O), 65.48 (C-O), and 34.18 (C-S) ppm; TLC and GLC as stated above.

2-Mercaptoethanol *O*-palmitate *S*-acetate (**4**). A solution of 2-mercaptoethanol (0.70 ml, 10 mmol), acetic acid (0.57 ml, 10 mmol), and DCC (2.08 g, 10 mmol) in 100 ml of methylene chloride was prepared at 0–5°C and allowed to stir for 2 hr attaining room temperature. Palmitic acid (2.56 g, 10 mmol) and DCC (2.08 g, 10 mmol) were added along with 200 ml of the solvent, and the resulting mixture was stirred for 16 hr. The mixture was suction filtered, the solvent was removed from the filtrate, and the crude product was then recrystallized from hexane with filtration. A recrystallization from methanol gave 1.54 g (44.3%) of the mixed diester: mp 47–49°C IR 1740, 1690, 1050  $cm^{-1}$ ;  $^{13}C$  NMR 195.0 (SC=O), 173.56 (OC=O), 62.51 (C-O), 44.11 (C-S), and 34.18 (S[C=O]CH<sub>3</sub>) ppm; GC-MS 239 ( $C_{15}H_{31}$  C=O)<sup>+</sup>.

2-Mercaptoethanol *O*-acetate *S*-palmitate (**5**). The S-palmitate (1.00 g, 3.16 mmol) was allowed to react with an equivalent amount each of DCC and acetic acid as above. After the usual workup procedure, the crude product was recrystallized from hexane, giving 1.01 g (89.4%) of the mixed diester isomeric with **4**: mp 33°C; IR as above;  $^{13}C$  NMR 198.69 (SC=O), 170.76 (OC=O), 62.90 (C-O), 44.11 (C-S), and 20.04 (O[C=O]CH<sub>3</sub>) ppm; GC-MS 239 ( $C_{15}H_{31}$  C=O)<sup>+</sup>.

## RESULTS AND DISCUSSION

Evaluation of the ratio of O- to S-acylation was obtained most easily from a set of IR spectral standards in  $CHCl_3$  with the pure O- and S-palmitates of 2-mercaptoethanol. Although separation of the two monoacylated, and the diacylated esters was easily achieved by HPLC, a solvent gradient was required, which relegated detection to UV. However, the extinction coefficient of the O-ester at 230 nm, for example, was more than thirty times that of the S-ester. This made estimation of the ratio of the products difficult when the S-ester was the minor component. We were unable to separate the monoesters by reverse phase HPLC (the increased solvent polarity that was required to affect separation, namely mixtures of acetonitrile and methylene chloride, caused the material to deposit in the column), and the monoesters rearranged in the injection port during gas chromatography. The IR analysis, fortunately, allowed reasonably accurate determination of the relative amounts of O- and S-ester (diester) present—ca. 2%.

The procedure for esterification described by Renard *et al.* (**3**) was repeated with palmitoyl chloride and gave a crude product that was 75% O-acylated and contained about 10% of the diester. Changing the reaction conditions (temperature and time) had no significant effect on this ratio. Reaction of palmitic acid with dicyclohexylcarbodiimide (DCC) in methylene chloride (Fig. 1), however, produced >97% S-acylation with the diester as the only contaminant detectable by TLC. Evidently the greater nucleophilicity of the thiol group is manifest in the absence of base under conditions whereby neutral oxygen and sulfur compete for the acylated imide (or palmitic

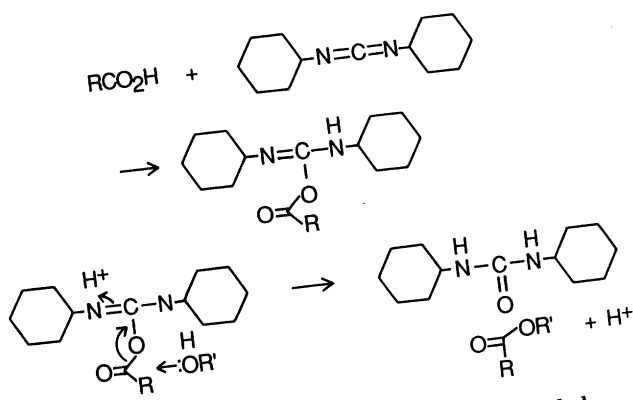


FIG. 1. Syntheses of S- and O-esters of mercaptoalcohols.

anhydride). When this procedure was employed using stearic acid, the relatively insoluble stearic anhydride precipitated, and longer reaction times were required. The addition of a catalytic amount of 4-dimethylaminopyridine (DMAP), facilitated the reaction, and was thereafter routinely employed for the sake of a uniform procedure. The combination of DMAP and DCC to secure thiol esters from thiols has been previously reported, although it was not ascertained that the reaction would be selective for thiols in the presence of alcohols (7). Interestingly, the alkylation of 2-mercaptoethanol with one equivalent of butyllithium and palmitoyl chloride in tetrahydrofuran at  $-78^{\circ}\text{C}$  produced a crude material that was 79% O-acylated, while the same reaction conducted with the addition of two equivalents of hexamethylphosphoric triamide (sufficient to tie up the lithium cation, presumably) produced material that was 94% S-acylated. Since the procedure with DCC was easier to conduct and employed the organic acid itself as the reagent, it appears to be the method of choice.

We then turned our attention to the problem of transacylation. Exposure of 2-mercaptoethanol S-palmitate to the assay conditions of Renard *et al.* (3), namely 20% HMPT in water at pH 8.0 and at  $30^{\circ}\text{C}$ , led to 5% transacylation in 18 hr. Since the assay is conducted during

the course of a few minutes, migration of acyl groups from S to O would not affect the assay significantly. Simple stirring of a solution of the S-ester in either HMPT or pyridine for 24 hr at  $30^{\circ}\text{C}$  similarly produced no more than 5% of O-ester. However, exposure of that ester to an equal weight of silica gel as a suspension in ether for 16 hr gave 70% O-ester, while Florisil produced 80% rearranged ester under the same conditions. Evidently, column chromatography on silica gel of S-esters that feature a vicinal hydroxyl group exposes the compound to potential isomerization. Treatment in ether with an equivalent of *p*-toluenesulfonic acid hydrate, *p*-TsOH.H<sub>2</sub>O, yielded predominantly O-ester (93%), and much of the remaining product was the free acid. Rearrangement to the O-ester, for synthetic purposes can be accomplished by warming the S-ester in benzene with a catalytic amount of anhydrous *p*-TsOH (Fig. 1).

Selectively diacylated materials can be prepared easily from 2-mercaptoethanol, using the selectivity of the DCC-promoted acylation, and then depending on a relatively fast O-acylation that employs a different acid and is not complicated by S to O migration. In this manner, the isomeric acetate-palmitates, 4 and 5, were prepared, the <sup>13</sup>C NMR spectra of which were distinguishable and clean.

#### ACKNOWLEDGMENTS

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